A COMPARATIVE EVALUATION OF 3 DIFFERENT REMINERALIZING AGENTS ON ARTIFICIALLY CREATED SURFACE CARIOUS LESION - INVITRO STUDY

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In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH - IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

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CONTENTS

INTRODUCTION

INTRODUCTION

Dental caries is one of the major causes for tooth loss in all age groups and affects both primary and permanent teeth. Dental caries is a dynamic demineralization and remineralisation process. [1] Cavitations occur when demineralization process is ahead of remineralisation process with initial carious lesion appearing as a white spot lesion. [2]

Saliva and gingival crevicular fluid mainly contain calcium, phosphate, and fluoride ions. The presence of these minerals at neutral pH helps in maintaining equilibrium condition between the mineral content of tooth and oral fluids. However when there is a drop in the oral fluid pH (below 5.5), dissolution of hydroxyapatite (HA) crystals and release of calcium and phosphate ions from tooth surface into oral fluids happen which is referred as demineralization. [3]

The demineralization process can be stopped by creating an environment conductive for remineralisation by various remineralising agents.[4] The process of restoring lost mineral ions in the tooth structure and strengthening the lattice work is known as remineralisation.[5]

The remineralised enamel crystallites are generally more resistant to decalcification and also have the same orientation as the original enamel crystallites [6]. The early enamel lesions have a potential for remineralisation with an increased resistance to further acid challenge, particularly with the use of enhanced remineralisation treatments [7]. Thus invasive treatments of pre cavitated lesions are not required. Various remineralising agents like fluoride [8], Casein phosphopeptide, stabilized amorphous calcium phosphate(C-AC Recaldent) [9, 10], unstabilized AC, Enamelon, CPP stabilized amorphous calcium phosphate with fluoride (CPP-ACPF Recaldent) (11,12), has been studied in both in-vitro (13-15) & in-vivo studies (16,17) .

Remineralising agents are available in various forms such as restorative materials, pit & fissure sealants, chewing gums, mouth rinses and dentifrices.(18,19) One of the most effective remineralising agents in caries prevention is fluoride. Nevertheless, some concerns have been expressed about fluorosis and total fluoride intake.(18,19) In recent years, fluoride alternatives have been proposed, which include CPP-ACP and nano-hydroxyapatite (NHA) because of their anticariogenic characteristics.(20,21)

The modern dental practice is focused on prevention and minimal intervention; replacement of lost tooth substance with a bio mimetic material is considered as one of the fundamentals of minimal intervention dentistry. Several methods were introduced to remineralise an early tooth structure loss. (22, 23)

Nano hydroxyapatite (n-HAp) is one of the most biocompatible and bioactive materials with marked affinity to the enamel surface. These nano-sized particles are similar to the apatite crystals of tooth enamel in morphology and crystal structure. [24] Having better affinity for hydroxyapatite crystals of enamel.

NHA toothpastes were first investigated in Japan in the 1980s. Studies have reported more or comparable remineralising effects for NHA toothpastes in comparison to other toothpastes containing amino fluoride and fluoride. (25,26) Daily tooth brushing with NHA toothpaste can provide adequate amounts of HA and enrich the saliva and dental plaque to prevent the progression of initial caries.(27)

In current practice, Nano hydroxyapatite has been widely used as an effective anti caries agent mainly because of its unique potential to bring about remineralisation. [28] The size of the calcium phosphate crystals also plays an important role in the formation of hard tissue and also has a significant impact on its intrinsic properties, solubility & biocompatibility. [29]

Calcium sodium phosphate silicate bioactive glass (BAG) (Novamin) is another material introduced to aid in remineralisation. Calcium sodium phospho silicate disintegrates and gives off sodium that gets exchanged with hydrogen cations ($H+$ or $H3O+$) when it comes in contact with saliva, which results in the release of calcium ($Ca^{2}+$) and phosphate ($PO4^{2}$) ions from the particle structure. [30-32]

The result of transient increase in pH that brings about the precipitation of calcium and phosphate ions from the saliva, to form a calcium phosphate layer on the tooth surfaces. Ca-P complexes crystallize to form a hydroxy carbonate apatite that is chemically and structurally similar to biological apatite. [30, 33]

Bioactive glass when contacts with saliva, rapidly releases sodium, calcium and phosphorous ions into saliva that are available for remineralisation of the tooth surface. These ions are released form hydroxycarbonate apatite (HCA) directly. They also attach to the tooth surface and continue to release ions and remineralise the tooth surface after the initial application. These particles have been shown to release ions and transform into HCA for up to 2 weeks. Ultimately, these particles will completely transform into HCA. [34]

Novamin adheres to exposed dentin surface and forms a mineralized layer that is mechanically strong and resistant to acid. [35]

Tri Calcium Phosphate (TCP) is a new hybrid material created with a milling technique that fuses beta tricalcium phosphate (ß-TCP) and sodium lauryl sulfate or fumaric acid. This blending results in a “functionalized” calcium and a “free” phosphate ions, designed to increase the efficacy of fluoride remineralisation [36, 37]. ß-TCP is similar to apatite crystals and possesses unique calcium environments capable of reacting with fluoride and enamel. As the phosphate floats freely, the exposed calcium environments are protected by preventing the calcium from prematurely interacting with fluoride. TCP provides catalytic amounts of calcium to boost fluoride efficacy and may be well designed to coexist with fluoride in a mouth rinse or dentifrice because it will not react before reaching the tooth surface [38]. When TCP finally comes in contact with the tooth surface and is moistened by saliva, the protective barrier breaks down, making the calcium, phosphate and fluoride ions which are present in the teeth. The fluoride and calcium then reacts with weakened enamel to provide a seed for enhanced mineral growth relative to fluoride alone.

Micro hardness tests are commonly used to study the physical properties of materials and they are widely used to measure the hardness of teeth [39-41]. This method is easy, quick and requires only a tiny area of specimen surface for testing.

AIM AND OBJECTIVES

AIM AND OBJECTIVE

AIM

To evaluate the remineralisation efficacy of three different novel remineralising agents namely - Bioactive glass, Tri-calcium Phosphate, Nanohydroxyapetite.

OBJECTIVE

To compare the efficacy of three different novel remineralising agents namely- Bioactive glass, Tri-calcium Phosphate, Nanohydroxyapetite on artificially created carious lesion using Vickers microhardness testing machine.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

King et al (2006) [42], prepared enamel blocks of extracted human third molars in an in vitro study to compare the efficacy of 10% NHA toothpaste, 900 ppm sodium fluoride and 900 ppm sodium monofluorophosphate (MPF) and the results showed that all the three agents remineralised enamel lesions and no significant difference was noted between their efficacy. Differences in the results might be explained by the use of polarized light microscopy and microradiography and the differences in the materials used.

EC Reynolds (2008) [43], The NovaMin TM technology is based on calcium sodium phosphosilicate bioactive glass which is claimed to release calcium and phosphate ions intra-orally to help the self-repair process of teeth. No published studies could be found supporting the remineralisation of enamel subsurface lesions in vitro or in situ. Furthermore, no published studies could be found showing an anticariogenic efficacy of NovaMin TM in animal models or other caries model systems or randomized, controlled caries clinical trials. This technology appears to be at a very early stage of development.

Huang et al (2009) [42], prepared 129 bovine enamel blocks to compare the efficacy of 1%, 5%, 10% and 15% NHA and sodium fluoride with using of Vickers microhardness tester and scanning electron microscopy and they found that optimal concentration (10%) of NHA resulted in remineralisation of initial enamel lesions.

HA, which is a bioactive and biocompatible material, is one of the primary components of tooth mineral content. HA is expected to significantly enhance remineralisation of initial enamel and dentin caries, and NHA is believed to have a higher efficacy than HA for this purpose due to its nano-size particles [11]. NHA has hydrophilic and wetting characteristics and is capable of producing a thin but tightly bound layer on the tooth surface, resulting in higher surface hardness and remineralisation. HA is capable of obstructing the dentinal tubules and thus, relieves tooth hypersensitivity. [13]

Chanya Chuenarrom et al (2009) [44], conducted a study to evaluate the effect of indentation loads and times on Knoop and Vickers microhardness tests for human enamel and dentin and they concluded that the difference of indentation times was not influential on KHN and VHN values of enamel and dentin for the same indentation loads but the KHN values of enamel and the VHN values of dentin were affected by variation of indentation loads.

HuangsB et al (2009) [45], conducted to determine the effect of nano-hydroxyapatite-containing toothpastes on initial enamel lesions under dynamic pH-cycling conditions and studies concluded that nano-HA has the potential to remineralise initial enamel lesions. A concentration of 10% nano-HA may be optimal for remineralisation of early enamel caries.

Huang in (2009) and Swarup in (2012) [46], used fluoride in combination with nanohydroxyapatite and concluded that nanohydroxyapatite produced a new surface layer over the demineralised enamel, with morphology similar to that of biologic enamel. They also stated that 10% nanohydroxyapatite is optimal for remineralisation of early carious lesions.

Braurer D et al (2009) [47], performed a study to understand the effect of addition of fluoride in the properties of bioactive glasses.CaF_2 concentration was increased in Sio_2-CaO-P_2O_5-NaO_5 system while network connectivity was kept constant. It was observed that incoporation of fluoride in bioactive glass, decreased it Tg which means that the glass has reduced hardness and is more bioactive. Also the onset of crystallization and peak temperatures were decreased when CaF_2 was increased.

S.B. Huang et al 2009, Shengbin et al 2010 and S. Huang et al 2011 **[48]**, reviewed the surface chemical properties and morphological structure of hydroxyapatite has been claimed to play the most important part in re-mineralization of early caries lesions. Three studies showed significant improvement in surface microhardness post treatment with nano-hydroxyapatite toothpaste similar to a review by **Kim et al 2007**.

Itthagarun et al 2010, Peter et al 2011 **[49]**, has shown that lesions can be re-hardened by deposition of hydroxyapatite that is initially deposited near the surface layer of the enamel and this was found to be significant. Future studies are required for further assessment of the potential of nano-hydroxyapatite toothpaste on enamelin-vivo.

Peter et al (2011) **[50]**,evaluated the effects of nano-hydroxy apatite (n-HAp)tooth pastes on remineralization of bovine and dentine subsurface lesions and they concluded that tooth pastes containing nano-hydroxy apatite revealed higher remineralising effects compared to amine fluoride toothpastes with bovine dentine, and comparable trend were obtained for enamel.

Arathi rao et al(2011)[51], reviewed a concentration of 10% nanohydroxyapatite is optimal for remineralisation of early enamel caries.[27] and hydroxyapatite has been used in toothpastes (as fillers) and pit-and-fissure sealants. and also the crystals of hydroxyapapite can effectively penetrate the dentin tubules and obturate them and can cause closure of the tubular openings of the dentin with plugs within 10 minutes as well as a regeneration of a surface mineral layer.

Tschoppe et al (2011) [52]**,** prepared 85 dentin blocks of bovine teeth to compare the efficacy of 7%, 20% and 24% NHA toothpastes and 0.14% amine fluoride by using microradiography and they found that NHA toothpastes had greater efficacy for remineralisation of initial lesions compared to amine fluoride.

Najibfard et al (2011) [53]**,** compared 10% and 5% NHA and a combination of 10% NHA + 1100 ppm NAF in an in vivo study based on microradiographs, the results showed comparable remineralising effect of the toothpastes evaluated. This finding is in contrast to our findings, which might be attributed to differences in concentration of NHA and study designs. We used Vickers microhardness tester but Najibfard et al evaluated the remineralising effect with microradiography. Differences in the results might be explained by the use of polarized light microscopy and microradiography and the differences in the materials used.

Tschoppe P et al (2011) [54]**,** conducted a study on Enamel and dentine remineralisation by nano-hydroxyapatite toothpastes concluded that toothpastes containing nano-hydroxyapatite revealed higher remineralising effects when compared to amine fluoride toothpastes.

Yuan et al (2012) [55]**,** used 48 dentin specimens to compare the efficacy of 3% NHA and conventional toothpastes. Based on energy-dispersive spectrometer

(EDS), they concluded that NHA was highly capable of obstructing the tubules and remineralising the tooth structure, which is in accord with our study results.

J.Shanti Swarup, Arathi Rao (2012) [56],conducted a study about to explore the effects of synthetically processed nanosized biomimetic HA particles in causing remineralisation of the early enamel lesion in comparison with 2%sodium fluoride & they concluded that 10% biomimetic nanohydroxyapatite of the particle size 20nm has the potential to remineralise initial enamel caries under in vitro conditions when compared with 2% sodium fluoride. This documented biomimetic apatite coating on the demineralised enamel suffices the need for a synthetic enamel biocompatible material able to repair early enamel lesions. nano hydroxyapatite would therefore be beneficial in promoting remineralisation with regular daily usage.

Goswami M, Saha S1, Chaitra TR (2012) [57], done a study to determine the effect of nano-hydroxyapatite concentrations on initial enamel lesions under dynamic pH-cycling conditions. It was concluded that nano-hydroxyapatite had the potential to remineralise initial enamel lesions. A concentration of 10% nano-hydroxyapatite may be optimal for remineralisation of early enamel caries.

Somkamol Vanichvatana and Prim Auychai (2013) [58], compared the efficacy of CPP–ACPF and fTCP (calcium phosphate pastes) with that of conventional 0.1%fluoride toothpaste in remineralising enamel on artificial caries lesions and they concluded that all three groups remineralised the enamel slab lesions, indicating model sensitivity to fluoride. Given the differences in usage amounts and treated regimens, Clinpro Tooth Creme provided similar benefits to the 0.1% fluoride toothpaste, however, no additional benefit of Tooth Mousse Plus was observed when used in conjunction with the 0.1% fluoride toothpaste.

Tabari M et al. (2013)[59], prepared nano-hydroxyapatite preparation by in-situ hybridization method and found that application of nano-hydroxyapatite preparation increased microhardness of tooth whether applied before or after iron drop exposure, but results were found better after the application of iron drop exposure. Application of nanohydroxyapatite preparation in our study also showed increase in microhardness of enamel after iron drop exposure.

Balakrishnan A et al (2013) [60], evaluated the remineralisation potential of various dentifrices over a period of 30 days and concluded that the extent of remineralisation achieved was dose dependant and increased with increasing the time of exposure and duration of the study .

Namrata Patil et al (2013) **[61],** compared the remineralising potential of three agents (CPP-ACP, CPP-ACP & fluoride and tricalcium phosphate & fluoride) on artificial enamel carious lesion and they concluded All the three remineralising agents in the study could effectively remineralise artificial enamel caries and they reported that TCP & fluoride-based products performed better than CPP-ACP-based products in remineralising artificial enamel caries.

Sai Sathya Narayana et al (2014) [62], Remineralisation efficiency of bioactive glass on artificially induced carious lesion – in vitro and concluded that bioactive glass is an effecive remineralising agent. NovaMin® : It is the trade name for a calcium sodium phosphosilicate bioactive glass, which is originally developed for the treatment of hypersensitivity by the physical occlusion of dentinal tubules. **[7]**

Haghgoo et al (2014)[63], found no differences between NHA and NaF mouthwashes in remineralising effect. However, surface microhardnes and tooth remineralisation significantly increased, and they used the remineralising agent in the form of a mouthwash.

Su-Yeon Jo et al (2014) [64], examined the effects of fluoridated, casein phosphopeptide–amorphous calcium phosphate complex (CPP-ACP)-containing, and functionalized β-tricalcium phosphate (fTCP) containing toothpastes on remineralisation of white spot lesions (WSLs) by using Quantitative light-induced fluorescence (QLF-D) Biluminator TM and they concluded that fTCP- and CPP-ACP containing toothpastes seem to be more effective in reducing WSLs than 1,000ppm fluoride-containing toothpastes.

De Carvalho et al(2014)[65] , reported that nano-HAP group showed significantly higher difference in surface hardness recovery after using the same pH cycling method as this study than fluoride varnish. This may be attributed to the number of applications of the nano-P paste, where it was applied for a total of seven times, while the fluoride varnish was applied only once. Taking into consideration the difference in the assessment method as they used KNH, it can be deduced that increasing the times of application of the nano-P paste could enhance the effect of remineralisation.

Sai Sathya Narayana et al (2014)[66], Investigated the efficacy of bioactive glass containing product on remineralisation of artificial induced carious enamel lesion and to compare its efficiency with other remineralisation products using an in-vitro pH cycling method and they concluded that Each test group when compared with the control group showed a significant difference existing for element Ca and P. and SHY-NM showed an increase of calcium suggesting that bioactive glass can be considered as an effective remineralising agent and F increase was seen in Amflor followed by CPP-ACPF as both contained fluoride.

Mehta et al (2014) [67],showed that bioactive glass (Novamin) and casein phosphopeptide-amorphous calcium phosphate successfully remineralised early enamel caries. However Novamin remineralised the carious lesion more effectively and CPP-ACP had an amorphous nature and couldn't properly adhere to the enamel surface. This also led to lower hardness value for CPP-ACP while Novamin showed higher values of hardness because it attached to the surface more compactly.

Adit Bharat Mehta et al (2014) [68],evaluate and compare the remineralisation potential of bioactive-Glass (BAG) (Novamin®/ Calcium-sodium-phosphosilicate) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing dentifrice and they concluded that both BAG and CPP-ACP are effective in remineralising early enamel caries. Application of BAG more effectively remineralised the carious lesion when compared with CPP-ACP.

Nithin m. g & joseph john (2015) [69],they has been done a systematic review was to evaluate how effective is the remineralisation potential of nanohydroxyapatite toothpaste on enamel and they concluded that the re-mineralizing potential of nano-hydroxyapatite toothpaste on enamel, the effectiveness of this toothpaste seems to be obvious in improving the initial enamel carious lesions.

Samuel B. Low et al (2015) [70],evaluated effectiveness of a commercially available toothpaste containing potassium nitrate, sodium monoflurophosphate, and nano-hydroxyapatite as well as antioxidants phloretin, ferulic acid and silymarin in reducing dental hypersensitivity in adults and they concluded that a toothpaste containing potassium nitrate, sodium monoflurophosphate and nano-hydroxyapatite plus antioxidants phloretin, ferulic acid and silymarin applied daily significantly decreased tooth pain of dentin hypersensitivity within a two-day and two-week time period.

Vyavhare et al(2015) and Swarup and Rao (2012) **[71]**, who studied the effect of adding synthetically processed nanoHAP to toothpaste in remineralising initial enamel lesion.

MohammadBagherRezvani et al (2015) **[74],** evaluate the effect of nano-tricalciumphosphate (n-TCP) and nanohydroxyapatite (n-HAP) on prevention of restaining of enamel after dental bleaching and they concluded that 10% n-TCP couldsignificantlymaintaintheresultantcolourandreconstructtheenamelstructureafterbleaching.

Amaechi et al (2015) **[72],** reported that effectiveness of n-HAp containing toothpaste (Apagard) to physically occlude dentin tubules as a surrogate measure of its ability to clinically relieve dentin hypersensitivity.

Udaya Kumar Palaniswamy et al (2016) **[73],** evaluate remineralising potential of bioactive glasses (BAGs) and amorphous calcium phosphatecasein phosphopeptide (ACP-CPP) on early enamel lesion and concluded that Both the remineralising agents tested in this study can be considered effective in repair and prevention of demineralization. BAG showed better results initially, but eventually both have similar remineralising potential.

Rithesh kulal et al (2016) [74],they conducted a study about to evaluate and compare the effects of three different desensitizing agents (15% nano hydroxyapatite crystals; 5% novamin and 8% proargin) on dentinal permeability and tubule occlusion in-vitro and they concluded that all the three desensitising agents were effective in the dentine tubule occlusion. In addition efficacy of nano-hydroxyapatite toothpaste was greater compared to the other desensitising agents.

Asghar Ebadifar et al (2017) [75],they have done a study to assess the effect of nano-hydroxyapatite (NHA) on microhardness of artificially created carious lesions and they concluded that toothpaste containing NHA was more effective than the toothpaste without NHA for the purpose of remineralisation.

Abhishek Singh et al (2017) [76],compare the relative efficiency of nanohydroxyapatite (n-Hap) Aclaim, n-HAp Apagard, Clinpro Tooth Crème and Colgate Total in remineralisation and they concluded that Aclaim and Apagard on daily application will provide maximum protection against enamel demineralization in orthodontic patients.

A Arvindkumar et al (2017) [77], compared and evaluated the caries preventive efficacy of aresin infiltrant, casein phosphopeptideamorphous (CPP-ACP) and nanohydroxyapatite(nano-HA) on white spot enamel lesion and concluded that the resin infiltrant showed higher caries inhibition potential and superior acid resistance than CPP-ACP and nano-HA.

Nilesh Rathi et al (2017) [78], evaluate and compare the microhardness of deciduous teeth treated with nano-hydroxyapatite and calcium sucrose phosphate after iron drop exposure and they concluded that Nanohydroxyapatite preparation and calcium sucrose phosphate have remineralising effect over teeth affected by acid challenge of iron drops, nanohydroxyapatite preparation showing better results than calcium sucrose phosphate.

Rakesh Mittal et al (2017) [(79)], reviewed NovaMin is the brand name of a particulate bioactive glass. NovaMin is technically described as an inorganic amorphous calcium sodium phosphosilicate (CSPS) material that was designed based on a class of materials known as bioactive glasses. It comprises SiO_2 (45%), Na_2O (24.5%), CaO (24.5%) and P_2O_5 (6%) [26]. NovaMin is claimed to release calcium and phosphate ions intraorally to help the self-repair process of enamel.

A silica-rich surface layer forms through polycondensation of hydrated silica groups on which precipitation of ions happens which crystallizes over time to form a hydroxyl-carbonate apatite. Although it is used extensively as a desensitizing agent reports also claim that the chemical reactions that promote apatite formation may enhance the remineralisation.

A novel 5,000 ppm fluoride dentifrice, Clinpro 5000, was recently introduced by 3 M ESPE. This 1.1% NaFsilica-containing paste containing an innovative functionalized tricalcium phosphate (fTCP) ingredient that, when evaluated in development formulations, has been shown to boost remineralisation performance relative to fluoride-only systems.

Umang Jagga et al (2018) [(80)], compared the remineralising efficacy of novamin, tricalcium phosphate and they concluded that both novamin and tricalcium phosphate were effective in remineralising the carious lesions.

Issa Daas et al (2018) [81], compared the effectiveness of nano-hydroxyapatite (nano-HAP) paste and fluoride varnish in remineralising initial enamel lesion in young permanent teeth and their ability to resist secondary caries under dynamic pH cycling quantitatively & qualitatively and they concluded that Nano-HAP paste showed promising long-term protective effect in terms of surface depositions and maintaining a smooth surface compared with fluoride varnish.

Niwut Juntavee et al (2018) [82], investigated the effects of nano-hydroxyapatite (NHA) gel and Clinpro (CP) on remineralisation potential of enamel and cementum at the cavosurface area of computeraided design and computer-aided manufacturing ceramic restoration and they concluded that NHA gel and CP were capable of remineralisation of the enamel and cementum.NHA was more capable in the remineralisation process than CP. NHA was extremely capable in the remineralisation process for enamel and cementum surrounding the margin of the computer aided design and computer-aided manufacturing ceramic.

MATERIALS AND METHODS

MATERIALS & METHODS

SOURCE OF SAMPLE

Sixty freshly extracted sound human permanent maxillary first premolars were collected from the Department of Oral and Maxillofacial Surgery, Vivekanandha Dental College for Women, Tiruchengode.

MATERIALS USED

- Self cure acrylic.
- Deionised water.
- Demineralising solution.
- Tri-Calcium Phosphate (Clin pro tooth paste)
- Nano-hydroxyapetite (Aclaim tooth paste)
- Bioactive glass (Novamin)
- Artificial Saliva.

Armamentarium:

- Universal incubator.
- Contra angle hand piece (NSK).
- Polishing cup.
- Vicker's microhardness testing machine.

S.NO	Remineralising agents	Manufacturer	COMPOSITION
1.	Tri calcium phosphate	3M ESPE	Water, Sorbitol, Hydrated silica, Glycerin, Polyethylene-polypropylene Glycol. Flavor, Polyethylene Glycol, Sodium lauryl sulfate, Titanium dioxide, carboxymethyl cellulose, sodium saccharin, sodium fluoride, tricalcium phosphate
2.	Nano-hydroxy apatite	Group Pharmaceuticals Ltd	Sorbital, Glycerin, Silica, Purified water, Hydroxyapatite, cocamidopropyl betaine, Hydroxyethyl cellulose, Titanium dioxide, Flavour, sodium saccharin.
3.	Bioactive glass	GlaxoSmithKline	Sodium fluoride, Glycerin,hydrated silica, calcium sodium phosphosilicate (NOVAMIN), cocamidopropyl betaine, sodium methyl cocoyl taurate, aroma, titanium dioxide, carbomer,sodium fluoride.

Method of collection of samples:

Sixty maxillary first premolars were collected from the Department of Oral and Maxillofacial surgery, Vivekanandha Dental College for Women, Tiruchengode, which were indicated for extraction for therapeutic purpose.

Infection Control protocol for the teeth collected for this study:

Collection, storage, sterilization and handling of extracted teeth were followed according to the guidelines and recommendations given by:

Occupational Safety and Health Administration (OSHA) and Centre for Disease Control & Prevention (CDC):

1. Handling of teeth was always done using gloves, mask and protective eyewear.
2. Teeth were cleaned of any visible blood and gross debris.
3. Distilled water was used in wide mouth plastic jars for initial collection.
4. Teeth were immersed in 10% formalin for 7 days, following which the liquid was discarded and the teeth were transferred into separate jars containing distilled water.
5. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles.
6. As and when the teeth were required, they were removed from the jars with cotton pliers and rinsed in tap water.

Exclusion Criteria:

Teeth with any visible caries, hypoplastic or white spot lesions or any fracture were excluded from the study.

Inclusion Criteria: Caries-free teeth, extracted for therapeutic purpose were included.

PROCEDURE

Removal of external residual tissues:

The selected teeth were stored in 10% formalin following extraction and calculus was mechanically removed using hand scalers.

Preparation of the samples:

Teeth were embedded in selfcure acrylic with exposure of buccal surface of enamel following which the samples were then stored in deionised water for one month.

Demineralization solution & artificial saliva Solution preparation:

Both Demineralization solution & Artificial saliva were prepared in the Department of Biochemistry, Vivekanandha Dental College For Women, Tiruchengode.

Composition of Demineralising solution:

- 2.2mM calcium chloride ($CaCl_{2}.2H_2O$)
- 2.2mM monosodium phosphate ($NaH_2PO_{4}.7H_2O$)
- 0.05M lactic acid.

The final pH was adjusted to 4.5 with 50% sodium hydroxide(NaOH).

Composition of Artificial saliva

- 2.200g/L gastric mucin
- 0.381 g/L sodium chloride (NaCl)
- 0.213 g/L calcium chloride ($CaCl_{2}.2H_2O$)
- 0.738 g/L potassium hydrogen phosphate ($K_2HPO_{4}.3H_20$)
- 1.114 g/L potassium chloride (KCl)

The final pH was adjusted to 7.00 at 37^{o}c with 85% lactic acid.

Demineralising solution was used to demineralise the surface of the samples by immersing them in a glass containers containing 50mL of the solution for a period of 48 hours at 37^{o}C using an incubator. This procedure was aimed at producing a consistent subsurface lesion, following which demineralization process for 48 hours.

After demineralisation process the samples were washed in deionised water and dried with air syringe.

Topical application

Samples were randomly divided into four groups consisting of 15 samples each as follows:

Group 1 - Tri-Calcium Phosphate (n=15).

Group 2 - Nanohydroxyapetite (n=15).

Group 3 - Bioactive glass (n=15).

Group 4 - Artificial Saliva (Control) (n=15).

The samples from each group were treated with the respective remineralising agent (except for the control group) for 4 min , at every 24^{th} hours for a period of 7 days with help of polishing cup attached to a contra-angle hand piece. The samples of control group was only placed in artificial saliva. The samples of experimental groups were rubbed with the respective remineralising agent for 4 minutes and placed in artificial saliva after washing with deionised water.

All samples were incubated in a universal incubator at 37^{o}C between each remineralising cycle. After 7 cycles of remineralisation, surface micro hardness of the specimens was determined using a Vicker's micro hardness testing machine (Anna University, chennai).

The micro hardness values were tabulated & compared to determine the remineralisation potential of remineralising agents used in this study.

FIG 1: PRE OPERATIVE SAMPLES

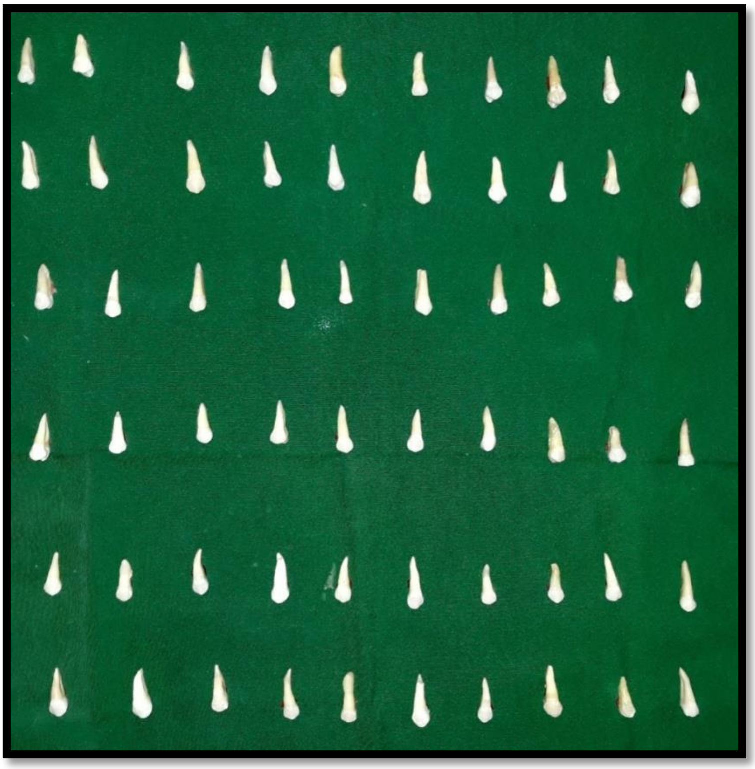


FIG 2: SAMPLES IMMERSED IN DEIONIZED WATER

FIG 3: SAMPLE EMBEDDED IN ACRYLIC RESIN

FIG 4: REMINERALISING AGENTS

FIG 5: VICKER' S HARDNESS MACHINE

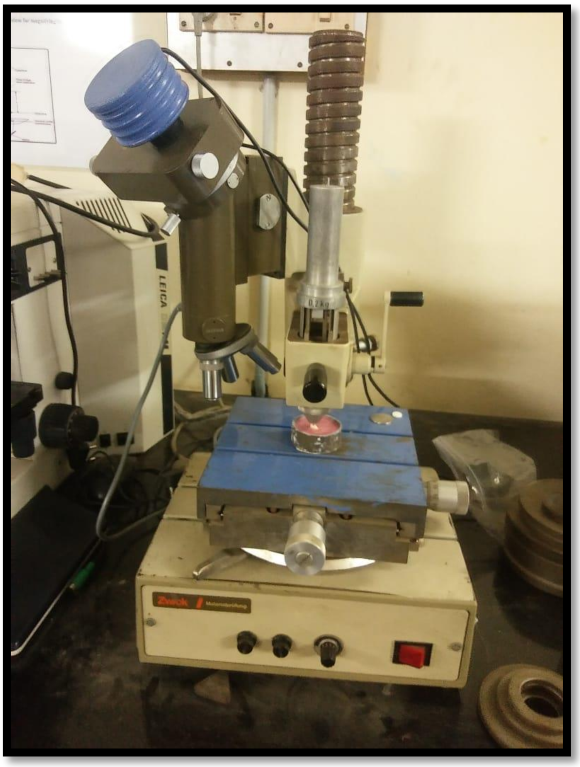


FIG 6:

FIG 7: SAMPLES IMMERSED IN DEMINERALISING SOLUTION

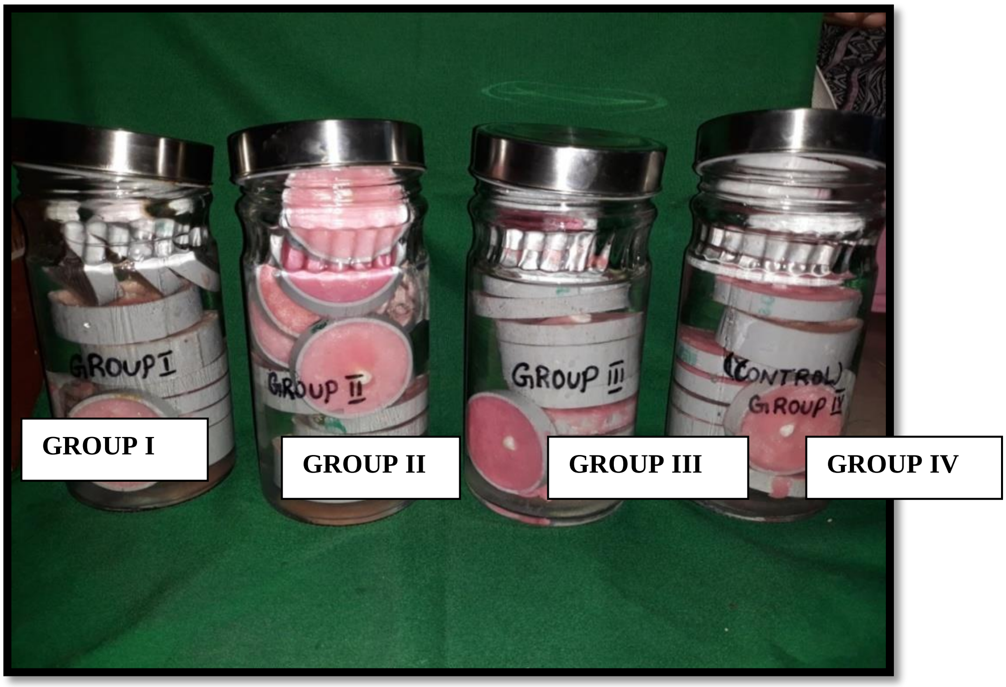


FIG 8: APPLICATION OF REMINERALISING AGENTS

FIG 9:

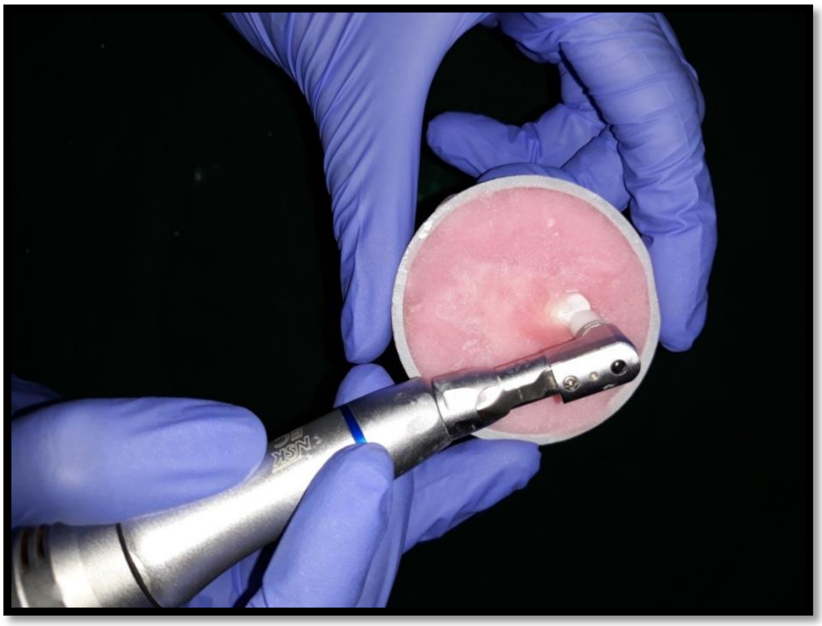


FIG 10: ARTIFICIAL SALIVA

FIG 11: DEMINERALISING SOLUTION

FIG 12: GROUP I

FIG 13: GROUP II

FIG 14: GROUP III

FIG 15: GROUP IV

FIG 16: DEMINERALISING SOLUTION REAGENTS

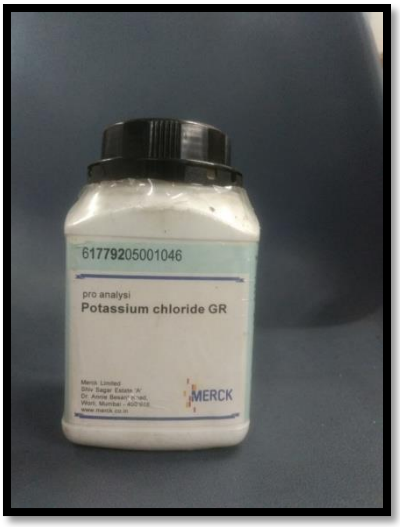
(a) Lactic Acid

(b)Calcium Chloride

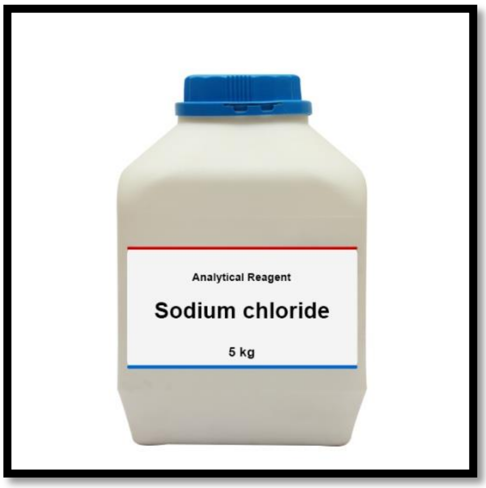
(c)Monosodium Phosphate

FIG 17: ARTIFICIAL SALIVA REAGENTS

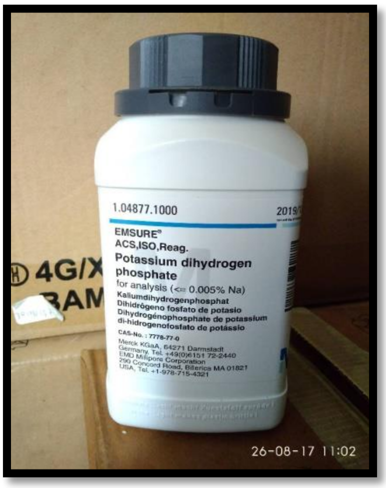
(a)Calcium Cloride



(b) Potassium Chloride



(c)Sodium Chloride



(d) Potassium Hydrogen Phosphate

RESULTS

RESULTS

GROUP I (TRICALCIUM PHOSPHATE)

CONSTANT	LOAD (P) (N)	AVG. DIA. LENGTH (D) (MM)	HV (MPA)
1.854	1.96133	0.158	145.662
1.854	1.96133	0.161	140.2842
1.854	1.96133	0.16	142.0432
1.854	1.96133	0.163	136.8627
1.854	1.96133	0.157	147.5235
1.854	1.96133	0.158	145.662
1.854	1.96133	0.158	145.662
1.854	1.96133	0.161	140.2842
1.854	1.96133	0.159	143.8344
1.854	1.96133	0.158	145.662
1.854	1.96133	0.157	147.5235
1.854	1.96133	0.162	138.5576
1.854	1.96133	0.159	143.8355
1.854	1.96133	0.161	140.2842
1.854	1.96133	0.160	142.0432

GROUP II (NANOHYDROXY APATITE)

CONSTANT	LOAD (P) (N)	AVG. DIA. LENGTH (D) (MM)	HV (MPA)
1.854	1.96133	0.143	177.8232
1.854	1.96133	0.142	180.3365
1.854	1.96133	0.141	182.9036
1.854	1.96133	0.140	185.5258
1.854	1.96133	0.141	182.9036
1.854	1.96133	0.144	175.362
1.854	1.96133	0.142	180.3365
1.854	1.96133	0.141	182.9036
1.854	1.96133	0.143	177.8232
1.854	1.96133	0.142	180.3365
1.854	1.96133	0.141	182.9036
1.854	1.96133	0.141	182.9036
1.854	1.96133	0.140	185.5258
1.854	1.96133	0.143	177.8232
1.854	1.96133	0.142	180.3365

GROUP III (BIOACTIVE GLASS)

CONSTANT	LOAD (P) (N)	AVG. DIA. LENGTH (D) (MM)	HV (MPA)
1.854	1.96133	0.202	89.11641
1.854	1.96133	0.199	91.82359
1.854	1.96133	0.198	92.75344
1.854	1.96133	0.196	94.65602
1.854	1.96133	0.199	91.82359
1.854	1.96133	0.200	90.90765
1.854	1.96133	0.201	90.00534
1.854	1.96133	0.195	95.62934
1.854	1.96133	0.197	93.69749
1.854	1.96133	0.198	92.75344
1.854	1.96133	0.198	92.75344
1.854	1.96133	0.196	94.65602
1.854	1.96133	0.197	93.69749
1.854	1.96133	0.202	89.11641
1.854	1.96133	0.201	90.00534

GROUP IV(CONTROL)

CONSTANT	LOAD (P) (N)	AVG. DIA. LENGTH (D) (MM)	HV (MPA)
1.854	1.96133	0.262	52.9734
1.854	1.96133	0.260	53.79151
1.854	1.96133	0.265	51.78079
1.854	1.96133	0.260	53.79151
1.854	1.96133	0.250	58.18089
1.854	1.96133	0.255	55.92166
1.854	1.96133	0.262	52.9734
1.854	1.96133	0.253	56.80929
1.854	1.96133	0.260	53.79151
1.854	1.96133	0.265	51.78079
1.854	1.96133	0.270	49.88074
1.854	1.96133	0.250	58.18089
1.854	1.96133	0.265	51.78079
1.854	1.96133	0.265	51.78079
1.854	1.96133	0.253	56.80929

STATISTICAL ANALYSIS

To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp. Released 2015) is used. Significance level is fixed as 5% ($\alpha = 0.05$).

TABLE 1

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal that the variable (HV) follows Normal distribution. Therefore, to analyse the data Parametric method is applied.

One-Way ANOVA To Compare Mean HV Values Between Groups:

Group	N	MeanHV (MPa)	Std. Dev	Std. Error of Mean	95% CI for Mean		p-value
					LB	UB	
Group -1	15	143.05	3.295	0.8507	141.22	144.87	<0.001
Group -2	15	181.05	2.963	0.7649	179.41	182.69	
Group -3	15	92.23	2.067	0.5337	91.08	93.37	
Group -4	15	54.02	2.582	0.6667	52.59	55.45	
Total	60	117.58	48.857	6.3074	104.96	130.21	

- The statistical parameters such as mean and SD of micro hardness of samples were obtained for each group as shown in Table 1. The mean for control was the lowest 54.02 ± 2.582 MPa. In case of materials used, NHA indicated highest mean 181.05 ± 2.963MPa, followed by TCP with a mean value of 143.05 ± 3.295 MPa, and bioactive glass with the least mean value of 92.23 ± 2.067 MPa [Table 1].

TABLE 2

ANOVA

Sum of Squares		df	Mean Square	F-value	p-value
Between Groups	140405.234	3	46801.745	6123.315	<0.001
Within Groups	428.019	56	7.643		
Total	140833.253	59			

- One-way ANOVA resulted into the F-statistic of 6123.315 with a corresponding P < 0.001.The test indicated statistically significant difference of mean microhardness across the groups. Accordingly, pair-wise comparison of microhardness was performed between groups to determine which groups differed significantly from other, with results shown in Table 2.

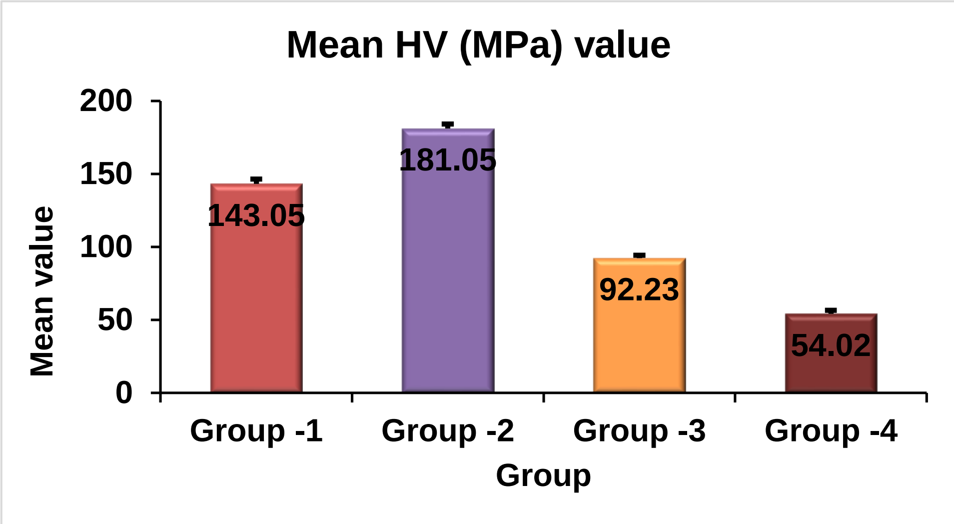
TABLE III

To compare the mean HV values between groups one way ANOVA is applied followed by Tukey's HSD post hoc tests for multiple pairwise comparisons.

Tukey Hsd Post Hoc Tests For Multiple Pair Wise Comparisons

Group		Mean Difference	p-value
Group -1	Group -2	-38.00	<0.001
	Group -3	50.82	<0.001
	Group -4	89.03	<0.001
Group -2	Group -3	88.82	<0.001
	Group -4	127.03	<0.001
Group -3	Group -4	38.21	<0.001

CHART



- The mean microhardness of Nano hydroxy apatite was higher than other two experimental groups; however, the difference was statistically significant as indicated by $P < 0.001$ while mean values for the three experimental groups were significantly different from each other.

DISCUSSION

DISCUSSION

Dental caries is a infectious microbiological disease of teeth that results in localized dissolution and destruction of calcified tissue(According to sturdevent) and WHO stated that dental caries is a post eruptive pathological process of external origin involving softening of the hard tooth tissue & proceeding to the formation of cavity. It is one of the most commonly occurring oral disease.

White spot lesions are the earliest macroscopic evidence of incipient caries where typically the enamel surface layer stays intact during subsurface demineralization [(83)]. At this stage without any intervention, it will eventually collapse into a full cavity [(84)].Near neutral pH of saliva has natural buffering capacity hence demineralisation of the tooth enamel is reversed by saliva in early stages. The components of saliva which includes calcium ions , phosphate ions, as buffering agents, fluoride ions and other substances are responsible for its buffering capacity[(85)].

Caries is a pH dependent process; as the salivary pH drops below 5.5 (critical pH) dissolution of enamel begins which is the first step in demineralisation process. This marks the beginning of early enamel caries[(86)]. In demineralization, the subsurface layer gets demineralised whereas the surface layer stays consistently unmutilated [(87)]. The process of demineralisation can be opposed by neutralising the oral pH which can be achieved by increasing the concentration of salivary calcium and phosphate ions. This process is termed as remineralisation which involves rebuilding of partly dissolved apatite crystals [(86)].

The strategy of aided remineralisation include direct delivery of ions to the site where and when they are needed the most [88] .Enamel is the most highly mineralized tissue in the body. It consists of microscopic crystals of hydroxapatite arranged in structural layers or rods, also known as prisms and surrounded by water.[89] Water and protein components in the tooth are important as they form channels for acids travel into the tooth and the minerals travel resulting in destruction of the tooth structure will occur .

The principal proteins involved in the hierarchical construction of enamel apatite crystals includes amelogenin, ameloblastins and proteniases . However; the proteins that induce or control the process of apatite crystallisation gets partially or completely degraded or removed during enamel maturation . Enamel, as a nonliving tissue is mainly composed of inorganic apatite(97 weight%), so even after substantial mineral loss also It can self repaired.

Prerequisites for Natural Remineralisation:

1. Calcium and Phosphate ions in saliva .
2. Blood rich in minerals, trace elements and vitamins.
3. Dentinal fluid.
4. Salivary pH .
5. Salivary flow .
6. Salivary proteins (90) .

Ideal requirements of remineralising agent includes:

- Should deliver calcium and phosphate ions into the subsurface.
- Should not deliver any excess of calcium.
- Should not favour calculus formation.

• Should work at an acidic pH so as to stop demineralization during a carious attack.

• Should work in xerostomic patients also, as saliva cannot effectively stop the carious process.

• Should be able to boost the remineralising properties of saliva.

• The novel materials should be able to show some benefits over fluoride.[100,101]

The goal of managing dental caries is to arrest the progression of demineralising lesion . Various materials have been used in clinical studies to prevent the dental caries. This study mainly focuses on evaluating the remineralisation efficacy of three newer novel materials namely- Bioactive glass ,Tricalcium phosphate, Nanohydroxyapatite.

Few decades ago, bioactive glass modified the functional capabilities of biomaterial from bio-inactive to bioactive by stimulation of strong response after implantation in the human body. (Example-osteoprotectivity).

A material to be classified as bioactive, it should have biological response that results in the formation of a strong chemical bond between the implanted material and soft / hard tissue. Certain composition of silicate-based glasses with calcium and phosphorous are in identical proportion to promote the growth of natural bone which can form such a strong bond without an investing fibrous layer.

Bioactive glass belongs to a group of biomaterial that are now widely used in the field of dentistry and orthopaedics. With the use of fluoride from conventional formulation the concomitant cariostatic mechanism can be explained by increasing the driving force for fluoroapatite.With widespread use of topical fluoride, the decrease in the incidence of dental caries have been experienced in the most industrialised countries which can be attributed largely to the preventive effective of fluoride.

BAG is an extensively studied biomaterial in the field of tissue engineering, bone regeneration and dentin remineralisation due to the remarkable capability of forming Hydroxycarbonate Apatite (HCA) [91,92] . Bioactive glass 45S5 (BAG) has been incorporated into dentifrices, desensitizing pastes and glass ionomer cements (experimentally). Although, it has been successfully proven that materials based on bioactive substance have the potential to promote remineralisation, only a limited number of studies have quantitatively monitored the remineralisation process.

The preventive role of fluoride is mainly due to the formation of calcium fluoride like precipitate the hampering of demineralisation, whilst fluoride level needed for remineralisation are assumed to be higher than those required to prevent lesion formation.

Another material is Tricalcium phosphate - When Tricalcium phosphate contacts with the tooth structure that is moistened with saliva, the protective barrier will break down, thereby making calcium, phosphate and fluoride ions available to induce remineralisation of demineralised surface. [99] Fluoride and calcium ions then react with weakend demineralised enamel to provide a substrate wherein enhanced mineral growth can be achieved.

Tricalcium phosphate (β-TCP), sodium laurylsulfate and fumaric acid are various form of tricalcium phosphate. Blending of these components result in the functionalised and free phosphate design to increase the fluoride remineralisation efficacy [36,37]. The apatite structure of tricalcium phosphate is similar to that of enamel thereby possessing the unique calcium ions into enamel capable of reacting with fluoride ions. When the phosphate ions flow freely, the exposed calcium environment are protected by preventing the calcium from prematurely interacting with fluoride.

Tricalcium phosphate effectively provide catalytic amount of calcium that boost the efficacy of fluoride and may be well designed to coexist with fluoride in a mouth rinse or dentifrice. Nanohydroxyapatite (nHAp) has gained widespread acceptance in the field of medicine and dentistry which is attributed to its biocompability and bioactivity when placed in contact with body tissue. Comparing the morphology and crystals structure the nano sized particle have similarity to the apatite crystals of tooth enamel.

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_6$ building block of enamel, are the one of the main structure of dental tissue representing the enamel and dentin about 95% - 97% wt and responsible for mechanical behaviour of dental tissues. Hydroxyapatite (HA) is the most stable form of calcium & phosphate ions. Enamel prismatic HA crystals consist of a weaving of prisms ranging from 3 to 5 μm in diameter. A single prism reveals a highly organized array of fastened needle like HA crystallites (approximately 30 nm thick, 60 nm wide, and several millimetres long). Unlike bone, in enamel and dentine when HA is dissolved or abraded, it cannot spontaneously remineralise because enamel is deprived of regenerative cells and contrarily dentine apposition occurs only towards the pulp tissues.[98]

In this study Group II showed the highest hardness value of 181.05 MPa for Nanohydroxy apatite followed by Group I (Tricalcium phosphate)- 143.05MPa Group III (Bioactive glass) - 92.23 MPa & Group IV-control (54.02 MPa).

Group IV showed the least hardness values compared to other groups (54.02 MPa).Group IV is control which is not treated with any remineralising agent. A goal of modern dentistry is to manage non cavitated caries lesions non-invasively through remineralisation in an attempt to prevent disease progression and improve aesthetics, strength, and function.

The main goal of this present study is comparing the ability of three novel remineralising agents which will help us to select a suitable material for remineralise the tooth. The present study shows that ability of nanohydroxy apatite tooth paste shows the superior remineralising efficacy to enamel and dentin lesion and also Tricalcium phosphate and bioactive glass also have the ability to remineralise the enamel and dentin but comparatively lower than the nanohydroxy apatite.

Carbonate hydroxyapatite nanocrystals having size, morphology, chemical composition and crystallinity comparable to that of dentine are said to remineralise the enamel.[26] A concentration of 10% nanohydroxyapatite is optimal for remineralisation of early enamel caries.[(102-105)] Hydroxyapatite has been used in toothpastes and pit-and-fissure sealants. Hydroxyapapite crystals can effectively penetrate the dentin tubules and obturate them and can cause closure of the tubular openings of the dentin with plugs within 10 minutes as well as a regeneration of a surface mineral layer.(2011, arathi rao).

The results showed significantly higher microhardness following application of all the three remineralising toothpastes. But Nanohydroxy apatite containing toothpaste exhibited a higher remineralising effect than fluoridated toothpaste.[(93)] Vickers microhardness test was used to evaluate demineralised and remineralised dental tissues, as it is a relatively simple, rapid, and non destructive method and has been previously used.[95,96]

Result shows that marked decrease in microhardness values after demineralization . Similar results were obtained by Diniz in 2009[97] who reported that the microhardness of bulk enamel was 286.77 VHN which reduced to 38.48 VHN after demineralization.

After remineralisation , there was a gradual increase in microhardness values in Enamel to 251.08 VHN at the 28th day which was similar to the control values (268.38 VHN). (Sullivan, 1995) reported on the ability of fTCP to react with fluoride at the enamel surface as well as penetrate into subsurface enamel lesions (Karlinsey et al., 2009a; Karlinsey et al., 2009b; Karlinsey et al., 2009c; Karlinsey et al., 2009d).

Similar to our results, Haghgoo et al (5) observed that nano-HA and novamin were effective for remineralisation on primary teeth. Vahid Golpayegani et al(4) found that novamin dentifrice has greater remineralisation effect than fluoride-containing dentifrices on carious like lesions.

The surface chemical properties and morphological structure of hydroxyapatite has been claimed to play the most important part in remineralisation of early caries lesions. Three studies showed significant improvement in surface micro hardness post treatment with nano-hydroxyapatite toothpaste (S.B. Huang et al 2009, Shengbin et al 2010 and S. Huang et al 2011) similar to a review by Kim et al 2007.[19] This resembles our present study results.

Evidences from the studies (Itthagarun et al 2010, Peter et al 2011) has shown that lesions can be re-hardened by deposition of hydroxyapatite that is initially deposited near the surface layer of the enamel and this was found to be significant. Future studies are required for further assessment of the potential of nano-hydroxyapatite toothpaste on enamel in-vivo.

Hydroxyapatite, a compound of calcium and phosphate, is a natural substance that makes up about 75 percent of the weight of dentin. It has excellent biological properties including non-toxic and non-inflammatory; and it has bio resorption properties under physiological conditions.

Most of the experimentation on hydroxyapatite has been with studies on remineralising enamel and on a lesser scale in dentin. Nano-hydroxyapatite crystals small enough to mimic the size of natural dentinal hydroxyapatite (20 nm) have been used to repair micrometer-sized tooth surface defects in vitro. The nano-crystals have been used in tooth pastes and mouth rinses to promote the repair of demineralised enamel or dentine surfaces.[19]. Some prophylactic products have been shown in vitro to fill micro defects at the etched enamel surface in as little as a 10 min application over the enamel and dentine surfaces.[18].

HA, which is a bioactive and biocompatible material, is one of the primary components of tooth mineral content. HA is expected to significantly enhance remineralisation of initial enamel and dentin caries, and NHA is believed to have a higher efficacy than HA for this purpose due to its nano-size particles.(115) NHA has hydrophilic and wetting characteristics and is capable of producing a thin but tightly bound layer on the tooth surface, resulting in higher surface hardness and remineralisation. HA is capable of obstructing the dentinal tubules and thus, relieves tooth hypersensitivity.(116)

In 2016 according to Rithesh kulal reported that the novel biomaterials nano-hydroxyapatite, novamin and proargin have different modes of action and produce varying degrees of obliteration of tubules on application and hence, vary in the amount of blockage of tubules. Nano-hydroxyapatite toothpaste was found to be the most effective in achieving dentinal tubule occlusion.

Resembling our present study, Pedreira de Freitaset al. compared the effect of 2% neutral NaF and nano-HAP after bleaching treatment and reported that the surface gloss increased only in the nano-HAP group.

Vickers microhardness test has been used in our study for assessing the microhardness of teeth on application of various remineralising agents. This test gives accurate readings in both soft and hard materials that means it gives an identical hardness numbers on similar materials at different loads(106) .The decalcified teeth lose the inorganic components leading to decreased hardness, but the accurate readings of this test made it our choice of analysis for microhardness.

There is no standard condition for enamel and dentine microhardness testing; therefore, selection of testing conditions depended on the researcher's decision. Numerous previous microhardness studies reported results of both KHN and VHN at different indentation loads and times (107-114). There are many reasons to perform tests at different conditions. A high load is chosen for the reason that it produces a large impression, and it is thus easy to measure the indentation diagonal. However, a high load applied on a soft surface causes an oversize indentation, where the diagonals are longer than the micrometer scale fitted to the eyepiece of the tester. Therefore in a pre-post experimental study of, for instance, enamel erosion, it is necessary to apply a small load for a comparison between the baseline surface and the eroded surface for the same indentation load.

Another possible reason for least values obtained may be due to the short treatment duration. Therefore, it is necessary to have a longer period of application to be able to detect deposition of calcium and phosphate ions in the demineralised lesion.

Seven-day remineralisation limited to remineralise artificial enamel caries completely. It is one of the drawbacks observed in the study. Hence, the period of application for complete remineralisation cannot be determined for all the remineralising agents used. Although surface remineralisation was confirmed, enamel subsurface remineralisation was not evaluated.[94]

Within the limitations of this in vitro study, one can infer that remineralisation takes place with the use of nano-HAP, tricalcium phosphate and bioactive glass. However, complete remineralisation did not occur within the time span of 7 days.

Our results shows that remineralising efficacy more in NHA toothpaste when compared to Bioactive glass and tricalcium phosphate toothpaste.

However, when using Vickers microhardness tester for the assessment of remineralising effect of agents, the researchers should be well aware of the limitations of this method and generalization of in vitro results to the clinical setting.

This method cannot completely simulate the oral conditions. Furthermore, this study evaluated the efficacy of a domestically made toothpaste containing NHA, which is more affordable than similar foreign products.

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

Summary

The objective of this study was to compare the efficacy of three different novel remineralising agents namely- Bioactive glass, Tri-calcium Phosphate, Nano hydroxyapetite on artificially created carious lesion using vicker's microhardness testing machine.

Sixty extracted human maxillary first premolars were collected and teeth were embedded in self cure acrylic with the enamel surface exposed and the samples were then stored in deionised water for one month. Demineralising solution was used to demineralise the surface of the samples by immersing them in a glass container containing 50mL of the solution, for a period of 48 hours at 37^{o}C using an incubator.

This procedure was aimed at producing a consistent subsurface lesion. After 48 hours of demineralisation, Samples were then randomly divided into four groups of 15 samples each according to the material used for remineralisation. In Group 1 - Tri-Calcium Phosphate (n=15),Group 2 -Nano hydroxyapetite (n=15),Group 3 - Bioactive glass (n=15), Group 4 - Artificial Saliva (Control) (n=15).The samples from each group were treated with respective remineralising agent (except for the control group) using with help of polishing cup attached to a contra-angle hand piece for 4 min, at every 24th hours for a period of 7 days. The control group samples were directly placed in artificial saliva.

The samples of experimental groups were rubbed with the respective remineralising agent for 4 minutes and placed in artificial saliva after washing with deionised water. All samples were placed in an universal incubator at 37^{o}C between each remineralising cycle. After 7 cycles of remineralisation, surface micro hardness of the specimens were determined using Vicker's micro hardness testing machine(Anna university, chennai). The micro hardness values of each group were compared to determine the micro hardness of the remineralising agent used.

Conclusion

- All the three remineralising agents showed improved surface remineralisation.
- Nano-hydroxyapatite (group II) comparatively performed better for remineralisation followed by Tricalcium phosphate (group I) and Bioactive glass (group III).
- However, complete remineralisation did not occur within 7 days.

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INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST

Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

No: VDCW/IEC/32/2016 Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. Sowmiya .T.

Title: A ComparativeEvaluation of 3 Different Remineralizing Agents on Artificially Created Surface carious lesion- Invitro Study.

Institutional ethics committee thank you for your submission for approval of above proposal .It has been taken for discussion in the meeting held on 25 .10.16.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

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